

RESEARCH ARTICLE

BIOTECHNOLOGY

***IN VITRO* MULTIPLICATION OF *GYMNEMA SYLVESTRE* R. BR. THROUGH NODAL EXPLANTS**

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ABSTRACT

Gymnema sylvestre R. Br. a very useful medicinal plant belongs to the family Asclepiadaceae. Nodal explants of *Gymnema sylvestre* were used for *in vitro* multiplication on a medium containing various concentrations of growth regulators such as BA and NAA. Among the combinations used, MS medium fortified with BA at 1.0 mg/l was found to be suitable for induction of multiple shoots. The shoots were observed within 1 week of inoculation. The regenerated shoots were transferred to rooting media supplemented with different concentrations of NAA. The highest percentage of rooting was observed with NAA at 1.0 mg/l. The regenerated plantlets were transferred to the field after acclimatization.

KEYWORDS

Gymnema sylvestre R.Br., nodal explants, *In vitro* multiplication, MS medium.

INTRODUCTION

Medicinal plants are nature's gift, which has curative properties due to the presence of various complex chemical substances of different composition. They are found as secondary plant metabolites in one or more parts of these plants. These secondary plant metabolites are grouped as alkaloids, corticosteroids, essential oils etc. according to their composition¹.

The use of herbs as medicinal plants due to the presence of various chemical substances is ever increasing. But since most of these plants are taken from the wild, hundreds of species are now threatened with extinction. To overcome this problem medicinal plants can be raised through a technique called plant tissue culture. It is a technique of growing plant cells, tissues and organs in an artificially prepared nutrient medium under aseptic condition².

Gymnema sylvestre R.Br. (Tamil – Sirukurinja) belonging to the family Asclepiadaceae, having lot of medicinal properties. It is distributed throughout the world and grows on plains as well as hills. The whole plant is used as antiperiodic, diuretic and stomachic. The roots are used as astringent, emetic, expectorant, refrigerant, stomachic and tonic. The stem acts as thermogenic, anti-inflammatory and as stimulating agent. The leaves are used to cure cough and fever. It is an important ingredient in Ayurvedic formulations for diabetes. Mixed with castor oil it is applied externally to swollen glands and to enlarged spleen. The seeds are used as emetic and remedy for cold³. The present paper is an attempt to multiply this important medicinal plant containing antidiabetic property, so that it yields a protocol for propagating method which leads to utilization of important bioactive compound in future.

MATERIAL AND METHODS

Healthy nodal cuttings of *Gymnema sylvestre* R.Br, each bearing four to five nodes were collected from mature plant growing in the medicinal plant garden, Holy Cross College (Autonomous), Tiruchirappalli, Tamilnadu, India. After removing the leaves, nodal segments were washed thoroughly under running tap water for 30 minutes, followed by treatment with Bavistin solution 1% for 15minutes. It was further washed under tap water thoroughly for pre-sterilization and then rinsed with distilled water thrice and finally surface sterilization was carried out for 3 minutes with 0.01% mercuric chloride (HgCl₂) and then explants were washed 3 times with sterile distilled water. After surface sterilization, the explants were inoculated on MS medium⁴ containing 3% (w/v) sucrose, various concentrations of cytokinin (BA)(0.5,1.0,1.5,2.0,3.0 and 4.0 mg/l) and auxin (NAA) (0.5,1.0,1.5 and 2.0mg/l). The pH of the medium was adjusted to 5.7 before gelling with 0.8% (w/v) agar and autoclaved at 121°C for 15minutes. Cultures were raised in 25×150 mm culture tubes plugged with non absorbent cotton. The cultures were incubated under 16 hours photoperiod and with light intensity of 3000 lux under cool white fluorescent lamps and 8 hours dark period and were observed at regular interval for the results.

RESULTS AND DISCUSSION

The nodal explants of *Gymnema sylvestre* R.Br. produced new shoot buds after 5 days of inoculation (Plate 1a, 1c). In the event of multiple shoot formation, new adventitious buds and shoots were developed from nodal region. Among the various concentrations (0.5, 1.0,1.5,

2.0, 3.0 and 4.0mg/l) of BA used, the best response was observed at 1.0 mg/l. A maximum number of 7 shoots (Plate 1b) was observed at 1.0 mg/l BA with the average length of 3.4 cm (Table 1, Fig -1). The multiple shoots were harvested and transferred to rooting medium with different concentrations of NAA (0.5 - 2.0 mg/l). A maximum number of roots (Plate 1d) was observed at 1.0 mg/l NAA with the average

length of 2cm (Table 1, Fig-1). BA is said to be the inducer of multiple shooting⁵. Similar findings of multiple shoot induction were observed in *Anogeissus pendula*⁶, *Aristolochia bracteolata*⁷ and *Enicostemma littorale*⁸. Our results of rooting coincide with the findings of others. Similar results were reported in *Mussaenda erythrophylla*⁹, *Baliospermum montanum*¹⁰ and *Garcinia indica*¹¹.

Table -1
Effect of different concentrations (0.5 - 4.0mg/l) of BA and NAA (0.5-2.0 mg/l) on multiple shoot formation and root induction from nodal explants of *Gymnema sylvestre* R.Br.

Hormone Concentrations (BA and NAA) in mg/l	Shooting with BA		Rooting with NAA	
	No. of shoots/explant Mean ± SD	Shoot length (cm) Mean ± SD	No. of roots/explant Mean ± SD	Root length (cm) Mean ± SD
0.5	4.0 ± 1.1	2.7 ± 0.57	-	-
1.0	7.0 ± 1.5	3.4 ± 1.1	2.1 ± 0.5	0.5 ± 0.3
1.5	4.0 ± 1.3	2.6 ± 0.4	2.7 ± 0.1	0.7 ± 0.6
2.0	2.0 ± 0.7	2.8 ± 0.8	3.0 ± 0.3	3.2 ± 0.2
3.0	2.0 ± 0.7	2.0 ± 0.6	-	-
4.0	2.0 ± 0.8	1.9 ± 0.65	-	-

Figure -1
Percentage of response to BA for shooting and NAA for rooting in *Gymnema sylvestre* R.Br.

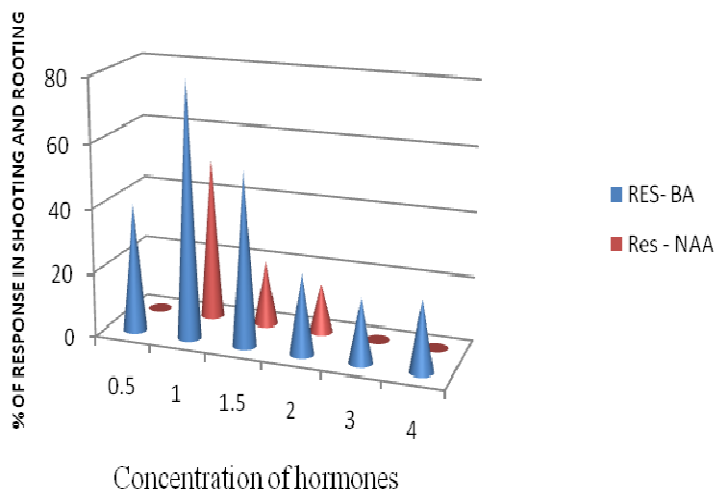


Plate 1

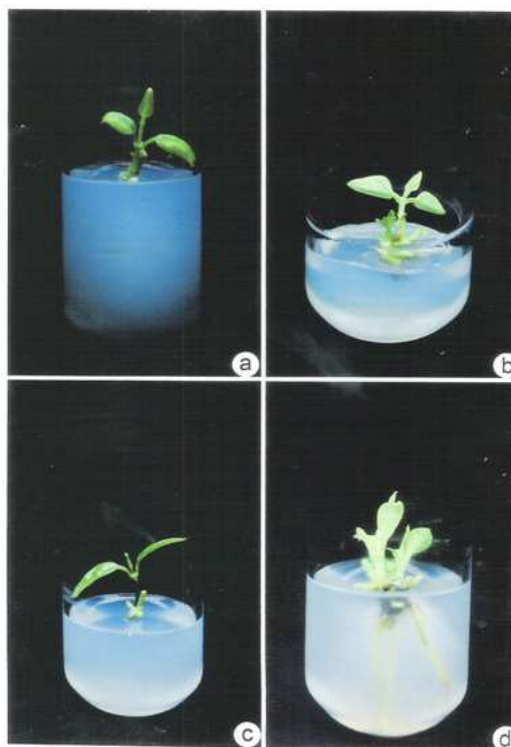
In vitro multiplication of *Gymnema sylvestre* R.Br. through nodal explants.

1a, 1c - Initiation of shoot buds from nodal explants of *G. sylvestre*

1b - Proliferation of shoots from nodal explants of *G. sylvestre*

1d - Rooting of regenerated shoots from nodal explants of *G. sylvestre*

Plate - 1



CONCLUSION

The present investigation indicates that growth and proliferation of multiple shoots in *Gymnema sylvestre* R.Br. can be stimulated easily by using

in vitro techniques. The method of multiple shoot formation described here may be used for rapid clonal propagation of *Gymnema sylvestre* and to overcome problems in conventional methods of propagation.

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